REMARKS

Claims 1-47 were pending in this application. Claims 1-14 and 33-47 have been withdrawn from consideration. Claims 15, 16, 18, and 20 have been amended to more distinctly claim and particularly point out that which Applicants regard as their invention. Claim 19 has been cancelled without prejudice. New claim 48 has been added. Support for new claim 48 can be found in lines 22-24 on page 20 and in lines 17-27 on page 29 of the instant application. Support for the amendments to the claims can be found in lines 16-17 on page 14. Further support can be found in lines 6-8 and lines 14-24 on page 33, in lines 20-29 on page 34, and on page 69, lines 17 through 25. Thus, as a result of the foregoing amendments, Claims 15-18, 20-32 and 48 remain for consideration.

The amended claims are shown above without markings. Attached hereto is a version with markings to show the changes made, captioned "Version with markings to show changes made."

No new matter has been added by way of the amendments. Reconsideration of this application is respectfully requested.

Claim Rejections under 35 U.S.C. §112, second paragraph

Claims 15-32 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Furthermore, the Examiner alleges that claims 15 and 16 are vague and indefinite in that the metes and bounds of the term "independent origin based cloning vector" or "IOBCV" are unclear.

Applicants respectfully traverse this rejection and refer the Examiner to page 33, lines 6 through 8, wherein "IOBCV" is defined as a nucleic acid insert which either is or contains a gene of interest. Furthermore, Applicants have also amended claims 15, 16, 18, and 20, to more distinctly claim and particularly point out that which Applicants regard as the invention and to further indicate the minimal structural/functional requirements for an "IOBCV" vector. Amendments to these particular claims would thus obviate the rejection of these claims as well as any dependent claims. In light of the foregoing, Applicants respectfully request withdrawal of the rejection.

The Examiner alleges that claims 15 and 16 are vague and indefinite in that the metes and bounds of the phrase "...wherein neither the IOBCV alone, nor the IOBCV in combination with the host cell can independently support homologous recombination..." are unclear. Examiner further alleges that it is unclear as to the particular conditions under which the vector alone or the vector plus host cell cannot support homologous recombination, and further questions whether the limitations only involve recombination with the IOBCV vector or whether it applies to any recombination done in the host cell.

Applicants respectfully traverse Examiner's rejections, and refer the examiner to page 7, lines 22 through 27 which states:

> "In a more intricate version of the present invention, the particular nucleotide sequence which has been selected to undergo homologous recombination is contained in an independent origin based cloning vector (IOBCV) that is comprised by the host cell, and neither the independent origin based cloning vector alone, nor the independent origin based cloning vector in combination with the host cell, can independently support homologous recombination."

Furthermore, Applicants refer the examiner to page 7, lines 27-29 and to page 8, lines 1-2, wherein Applicants state:

> "In a particular embodiment of this type both the independent origin based cloning vector and the host cell are RecA$^-$, and inducing the host cell to transiently support homologous recombination comprises inducing the transient expression of the RecA-like protein to support homologous recombination in the host cell."

Applicants respectfully point out that the present invention provides for a novel and efficient method of modifying independent origin based cloning vectors for either *in vitro* or *in vivo* gene expression. Moreover, as related to the instant application, since both the independent origin based cloning vector (IOBCV) and the host cell are RecA$^-$, neither the IOBCV alone, nor the host cell alone, nor the two in combination have the ability to produce a RecA-like protein to support homologous recombination. For homologous recombination to be successful in the present invention, transient expression of a RecA-like protein is necessary. This may be accomplished, for example, through use of a conditional replication shuttle vector that encodes a RecA-like protein. Support for this may be found on page 7, lines 6 through 12, on page 8, lines 2 through 6, and on page 34, lines 3 through 29 of the instant application.

Furthermore, Applicants point out that for purposes of the present application, methods are provided for selectively performing homologous recombination in a cell that normally cannot independently support homologous recombination. Thus, although one particular version of the present invention provides for the particular nucleotide sequence that has been selected to undergo homologous recombination to be contained by an independent origin based cloning vector (IOBCV) that is comprised by the host cell, this should not be construed to be a limitation on the scope of the invention as outlined in the instant application.

Moreover, Applicants have amended claims 15 and 16 to more distinctly claim and particularly point out that which Applicants regard as the invention. Support for the amendments can be found on page 34, lines 20 through 29, and on page 69, lines 17-25. Thus, withdrawal of the rejection is respectfully requested.

Fees

No fees are believed to be required, but if so, the Commissioner is hereby authorized to charge any fees, or credit any overpayment, to Deposit Account No. 11-1153.

Conclusion

Applicants believe that the foregoing amendments to the claims place the application in condition for allowance. Withdrawal of the rejections is respectfully requested. If a discussion with the undersigned will be of assistance in resolving any remaining issues, the Examiner is invited to telephone the undersigned at (201) 487-5800, ext. 118, to effect a resolution.

Respectfully submitted,

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Enclosure: VERSION WITH MARKINGS TO SHOW CHANGES MADE

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

15. (Amended) A method of selectively performing homologous recombination with a particular nucleotide sequence of [an independent origin based cloning vector (IOBCV)] a Bacterial or Bacteriophage-Derived Artificial Chromosome (BBPAC) that is contained in a recombination deficient host cell comprising introducing a conditional replication shuttle vector into a recombination deficient host cell and therein enabling homologous recombination in the host cell via the transient expression of a recombination protein in the host cell;

wherein the host cell comprises [an IOBCV] a Bacterial or Bacteriophage-Derived Artificial Chromosome (BBPAC) which contains the particular nucleotide sequence; wherein the conditional replication shuttle vector encodes a recombination protein that is transiently expressed by the host cell; wherein the conditional replication shuttle vector contains [a] homologous nucleic acid [that] sequences capable of selectively integrat[es]ing into the particular nucleotide sequence when the recombination protein is expressed; and wherein [neither the IOBCV alone, nor the IOBCV in combination with the host cell can independently support homologous recombination] the expressed recombination protein effectuates recombination of the shuttle vector and the Bacterial or Bacteriophage-Derived Artificial Chromosome (BBPAC).

16. (Amended) A method of selectively modifying a particular nucleotide sequence of [an independent origin based cloning vector (IOBCV)] a Bacterial or Bacteriophage-Derived Artificial Chromosome (BBPAC) that is contained in a recombination deficient host cell comprising:

(a) introducing a conditional replication shuttle vector into a recombination deficient host cell; wherein the host cell comprises [an IOBCV] a Bacterial or Bacteriophage-Derived Artificial Chromosome (BBPAC) that comprises a gene of interest which contains the particular nucleotide sequence; wherein the conditional

replication shuttle vector encodes a recombination protein that is expressed by the host cell and permits homologous recombination to occur in the host cell; wherein the conditional replication shuttle vector contains [a] homologous nucleic acid [that] sequences capable of selectively integrat[es]ing into the particular nucleotide sequence when the recombination protein is expressed thereby forming a co-integrate; wherein the nucleic acid sequences that selectively integrate[s] into the particular nucleotide sequence and the nucleic acid encoding the recombination protein are positioned on the conditional replication shuttle vector such that upon resolution of the co-integrate, the nucleic acid encoding the recombination protein remains with the conditional replication shuttle vector; and wherein [neither the IOBCV alone, nor the IOBCV in combination with the host cell can independently support homologous recombination] the expressed recombination protein effectuates recombination of the shuttle vector and the Bacterial or Bacteriophage-Derived Artificial Chromosome (BBPAC); and

(b) growing the host cell under conditions in which the conditional replication shuttle vector cannot replicate, therein diluting out the conditional replication shuttle vector encoding the recombination protein, and thereby preventing further recombination events in the recombination deficient cells.

18. (Amended) The method of Claim 16 wherein the conditional replication shuttle vector cannot replicate in the host cell because the conditional replication shuttle vector requires a particular protein for replication and neither the host cell nor the [IOBCV] Bacterial or Bacteriophage-Derived Artificial Chromosome (BBPAC) encode the particular protein.

20. (Amended) The method of Claim [19], 16 wherein the BBPAC is a BAC or a PAC.